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Diversity and seasonal fluctuation of tylenchid plant-parasitic nematodes in association with alfalfa in the Kerman Province (Iran)

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Abstract

The diversity and seasonal fluctuations of plant-parasitic nematodes (tylenchids) were investigated in five alfalfa fields in five counties of the Kerman Province of Iran during four consecutive seasons in 2013. Hundred soil samples were obtained per county, with 25 subsamples per field being composited to five to represent five replicates per field per county per season. In total, 500 samples were analyzed during the study. Nematodes were extracted from soil samples using the modified tray method. In total, 11 plant-parasitic nematode genera and 12 species were recorded. According to prominence values, *Ditylenchus* (*e.g. D. acutus*, *D. myceliophagus*, *D. terricolus* and *D. sarvarae*), followed by *Helicotylenchus pseudorobustsus*, *Pratylenchus neglectus* and *Meloidogyne javanica* were the most prominent species. The season and the localities significantly affect the population densities of nematodes. Values for both the Shannon (H') and Evenness (E) indices were the highest in Bam and the lowest in Jiroft counties. A significant, negative correlation existed between soil pH and mean population densities of *Scutylenchus rugosus*, while significant and positive correlations existed between soil electrical conductivity and *Helicotylenchus pseudorobustus*, *Aphelenchoides* sp., *Amplimerlinius globigerus* and *Pratylenchus neglectus*. In conclusion, diversity of plant-parasitic nematodes in Bam county was higher than other localities.

Keywords
Biodiversity, Nematode, Seasonal distribution.

Worldwide, alfalfa (*Medicago sativa* L.), a flowering plant belonging to the family Fabaceae, is cultivated as a forage crop (Tucak et al., 2008). It is the most important forage crop in Iran due to its superior feeding value for cattle, which is the main meat/protein food source (Tucak et al., 2008). The genus *Medicago* comprises many species, with up to 23 being cultivated in Iran (Ghanavati et al., 2007).

A wide range of plant-parasitic nematodes have been associated with alfalfa crops in various countries, such as the USA (Gray and Griffin, 1994), South Africa (Kleynhans et al., 1996) and others (Abivardi and Sharafeh, 1973; Sturhan and Brzeski, 1991). Moreover, nematodes such as *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* are major pests of the foliar parts of alfalfa (Gray et al., 1994; Milano de Tomasel and McIntyre, 2001), whereas *Meloidogyne* spp. and *Pratylenchus* spp. in particular infect roots of this genus and cause substantial yield losses (Hafez and Sundararaj, 2009). In Idaho (USA), *D. dipsaci* infections inflicted reduction in total yield of alfalfa ranging from 6 to 13% (Hafez, 1998). This author also reported yield reductions of between approximately 0.3 and 6% in the same study as a result of parasitism by *M. hapla*. *Ditylenchus dipsaci* is considered the most damaging plant-parasitic nematode that parasitize alfalfa in Iran

(Kheiri, 1972; Abivardi and Sharafeh, 1973). *Ditylenchus dipsaci* was the main cause of yield loss in alfalfa fields in the Khafr County, with stunted and infected plants being visible as patches of poor growth within the field (Kheiri, 1972; Abivardi and Sharafeh, 1973). Worldwide, nematode pests associated with roots of alfalfa include *Meloidogyne* spp., *Xiphinema* spp., *Pratylenchus* spp. and *Helicotylenchus* spp. (Hassanzadeh et al., 2004, Westerdahl and Frate, 2007; McCord, 2012). These nematode genera are also dominant in alfalfa fields in Iran.

Soil nematode communities represent superior biological tools for evaluating soil quality and plant health in terrestrial ecosystems (Wang et al., 2009; Pen-Mouratov et al., 2010). Nematode biodiversity in different soil habitats had been studied widely (Potter and McKeown, 2003; Biederman and Boutton, 2009; Zhang et al., 2012) as a crucial research component that gives an indication of soil quality (Bongers, 1990; Yeates, 2003; Neher et al., 2005). The latter is important for sustainable agriculture and also constitutes one of the main aims of an ecological study. Alfalfa is the main food source of domestic animals in the Kerman Province of Iran, which is in return the main income source of producers. Understanding distribution of most dominant plant-parasitic nematodes on alfalfa in Iran would provide better crop protection recommendations to alfalfa growers in Kerman Province, the main alfalfa production area, of Iran.

Hence, the aim of this investigation was to determine the biodiversity, prominence and seasonal population fluctuations of plant-parasitic tylenchids that occur in soil of alfalfa plants in this province and to identify whether relationships exist between selected soil properties and nematode population densities.

Materials and methods

Soil sampling

Five alfalfa fields in each of five counties, namely, Bam, Jiroft, Bardsir, Rabor and Rigan, were sampled for the presence of plant-parasitic nematodes during 2013 and 2014. Selection of the counties was based on the alfalfa production area of the province (Fig. 1; Table 1). Soil samples were collected four times during the year, namely, in October (Autumn), March (Winter), June (Spring) and August (Summer). In each field in each county, five discrete sub-samples were collected from each of five independent, 10 m × 10 m plots, representing five replicates and randomly chosen per field. The five sub-samples taken per field per plot were added together, mixed and one homogenized composite sample (representing one replicate) per plot ultimately obtained, totaling five replicates per field. In total, 500 samples (five composite samples per field × five fields × four seasons) were analyzed for nematode counts and identification. After removing the aboveground plant debris, soil samples were collected from the soil of alfalfa plants using a soil core with a 5-cm-diameter opening (Zhang et al., 2012).

Soil samples were stored in individual plastic bags, kept at 4°C and processed within one week after sampling. Additional soil was obtained during nematode sampling and used to analyze soil pH and electrical conductivity (EC) (Zhang et al., 2012) using standard methods (Rowell, 1994) (Table 1). Also, soil structure (% clay, % sand and % silt) was determined (Bouyoucos, 1962; Beretta et al., 2014). Average means for rainfall and temperature (Hashemi Nasab Khabisi et al., 2013; Kavian et al., 2016) as listed in Table 1, were used. The climate in the province ranges from dry and cold (Rabor and Bardsir counties) to warm and humid (Bam, Jiroft and Rigan counties) (Jalali-Far et al., 2012).

Nematode extraction and identification

Nematodes were extracted from 100 cm^3 composite soil samples over 72 hr using 40 × 25-cm plastic trays according to the modified Baermann tray technique (Whitehead and Hemming, 1965; Spaull and Braithwaite, 1979). The nematodes were counted with a stereomicroscope (Olympus CH-2; Japan) and their genera identification finalized using a light microscope (Nikon Eclipse E200). Nematodes were then fixed with a hot 4% formaldehyde solution and transferred to anhydrous glycerin (De Grisse, 1969) for species identification. The nematode genera were identified according to the classification (Brzeski, 1991; Andrássy, 2005; Castillo and Vovlas, 2007; Geraert, 2008, 2011). In addition, for accurate diagnosis of *Meloidogyne*, *Pratylenchus, Merlinius* and *Ditylenchus* species, DNA extraction was done using the Chelex method (Straube and Juen, 2013). The nematodes were identified using 28 S rDNA marker according to the protocol provided by Shokoohi et al. (2018).

Statistical analyzes

The relationships between nematode population density (MPD) and frequency of occurrence (FO) of each nematode genus identified were expressed as prominence value (PV) for each county and season. Ultimately, to determine which genera were

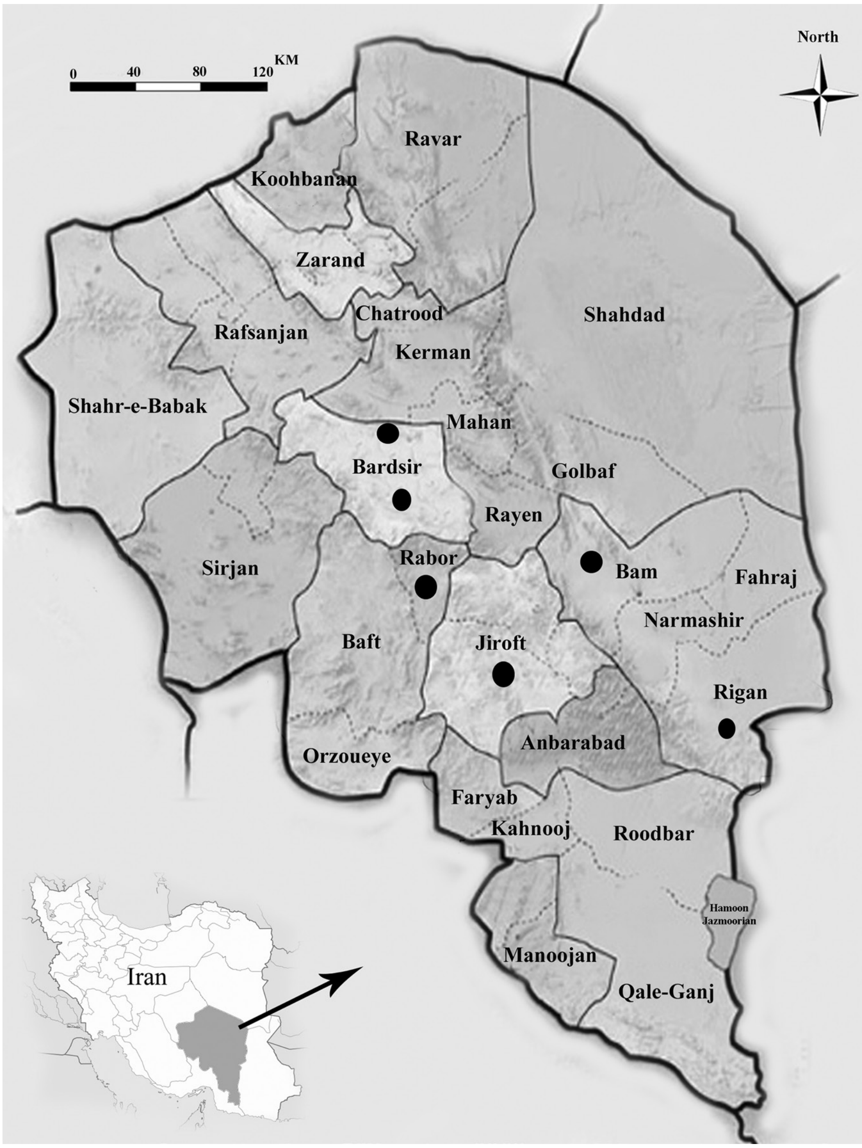

Figure 1: A map of the Kerman Province of Iran showing the five counties (indicated with black circles) where nematode samples were collected during four consecutive seasons during the 2013 seasons.

predominant in the Kerman Province in alfalfa bulk soil, population density data were pooled for each genus across the counties and over the four seasons and PV, MPD and FO again calculated using the equation PV = Population density × FO/10 (Bolton et al., 1989; De Waele and McDonald, 2000).

In addition, mean population densities for each nematode genus were log-transformed ($\log_{10}(x+1)$) and subjected to repeated Measures Analyses of Variance using SPSS Version 24 (IBM, 2016) to determine whether localities and/or seasons influenced abundance. Nematode biodiversity indices, representing the Evenness Index (E) (Zeng et al., 2012) and Shannon Index (H') (Colwell, 2009) were calculated. The E index refers to homogeneity of the species, whereas the H' index is the most popular biodiversity index used to summarize the diversity of a population to which each member belongs according to a unique group. The latter index also takes into account species richness and the proportion of each species within the community studied. Finally, correlations between nematode abundance and the two selected soil parameters, pH and EC were analyzed through two-tailed Pearson

Table 1. List of the localities sampled in the Kerman Province of Iran for the presence of nematodes, physical and chemical characteristics of the soils as well as rainfall and temperature data.

	Chemical soil characteristics					Temperature (°C) for the sampling period	
County	GPS coordinates	pH	EC (mhos/cm)	Soil type	Mean rainfall (mm) for the study period	Minimum	Maximum
Bam	N29°06′22″; E58°21′25″	7.53 ± 0.06	1.74 ± 0.2	Sandy	74	17	29
Bardsir	N29°55′39″; E56°34′20″	7.59 ± 0.08	2.97 ± 0.7	Sandy-loam	148	3	26
Jiroft	N28°40′41″; E 57°44′26″	7.53 ± 0.02	1.64 ± 0.06	Sandy-loam	155	17	33
Rabor	N29°17′28″; E56°54′47″	7.51 ± 0.03	2.04 ± 0.08	Sandy-loam	243	1	24
Rigan	N28°38′58″; E59°1′15″	7.59 ± 0.05	1.86 ± 0.04	Sandy	55	19	34

EC and pH data obtained from 20 samples per field ± SE. Mean of rainfall and temperature obtained from Hashemi Nasab Khabisi et al. (2013) and Kavian et al. (2016).

correlation using SPSS 24 (IBM 2016). Correlation of the localities with rainfall, temperature, pH and EC of the soil was done using XLSTAT (Addinsoft, 2007) through principal component analysis (PCA).

Results

In total, 11 plant-parasitic nematode genera (Table 1 or Fig. 2A-K) and 12 species were identified from 25 fields sampled from five counties. These included *Ditylenchus acutus*, *D. terricolus*, *D. myceliophagus* (Shokoohi et al., 2018), *D. savarae* (Shokoohi et al., 2018), *Helicotylenchus pseudorobustus*, *Meloidogyne javanica*, *Merlinius brevidens*, *Nanidorus minor*, *Pratylenchus cruciferus*, *P. neglectus*, *Amplimerlinius globigerus* and *Scutylenchus rugosus*. Unidentified species, *Aphelenchoides, Paratylenchus* and *Rotylenchus*, were also present.

The results indicated that locality and season effects significantly ($p \leq 0.01$) affected nematode community (Table 2). In terms of locality and seasonal fluctuation of nematode genera, substantial variation existed for MPD in different nematode genera over localities and four seasons (Table 3; Fig. 2A-K). According to PV, alfalfa fields in Bardsir were dominated by *H. pseudorobustsu* during the Winter (Table 3; Fig. 2B) and by *P. neglectus* during Summer (Table 3; Fig. 2C), whereas *M. brevidens* dominated in fields at Rabor during winter (Table 3; Fig. 2E). *Ditylenchus* dominated in alfalfa fields in Jiroft during Autum and Winter (Table 3; Fig. 2A) and *Meloidogyne* during Spring (Table 3; Fig. 2D). Interestingly, some genera were not detected at some localities and/or during some seasons. For example, *P. neglectus* was not present at Jiroft (Table 3; Fig. 2C), and neither *Meloidogyne* at Bardsir and Rigan (Table 3; Fig. 2D), *A. globigerus* at Rabor and Rigan (Table 3; Fig. 2F), *P. neglectus* at Jiroft (Table 3; Fig. 2H), *S. rugosus* at Bardsir, Rabor and Jiroft (Table 3; Fig. 2I), *Nanidorus minor* at Rabor and Jiroft (Table 3; Fig. 2J), and *Rotylenchus* sp. at Rabor, Bardsir, Jiroft and Rigan (Table 3; Fig. 2K). For seasons, the same phenomenon applied to *M. javanica* that were not detected during Autumn (Table 3; Fig. 2D), *N. minor* during Winter (Table 3; Fig. 2J), *Rotylenchus* sp. during Summer (Table 3; Fig. 2K) and *S. rugosus* during Autumn (Table 3; Fig. 2I) in all counties.

When nematode data were pooled for the five counties and four seasons, the predominant plant-parasitic nematode genera identified from the soil of

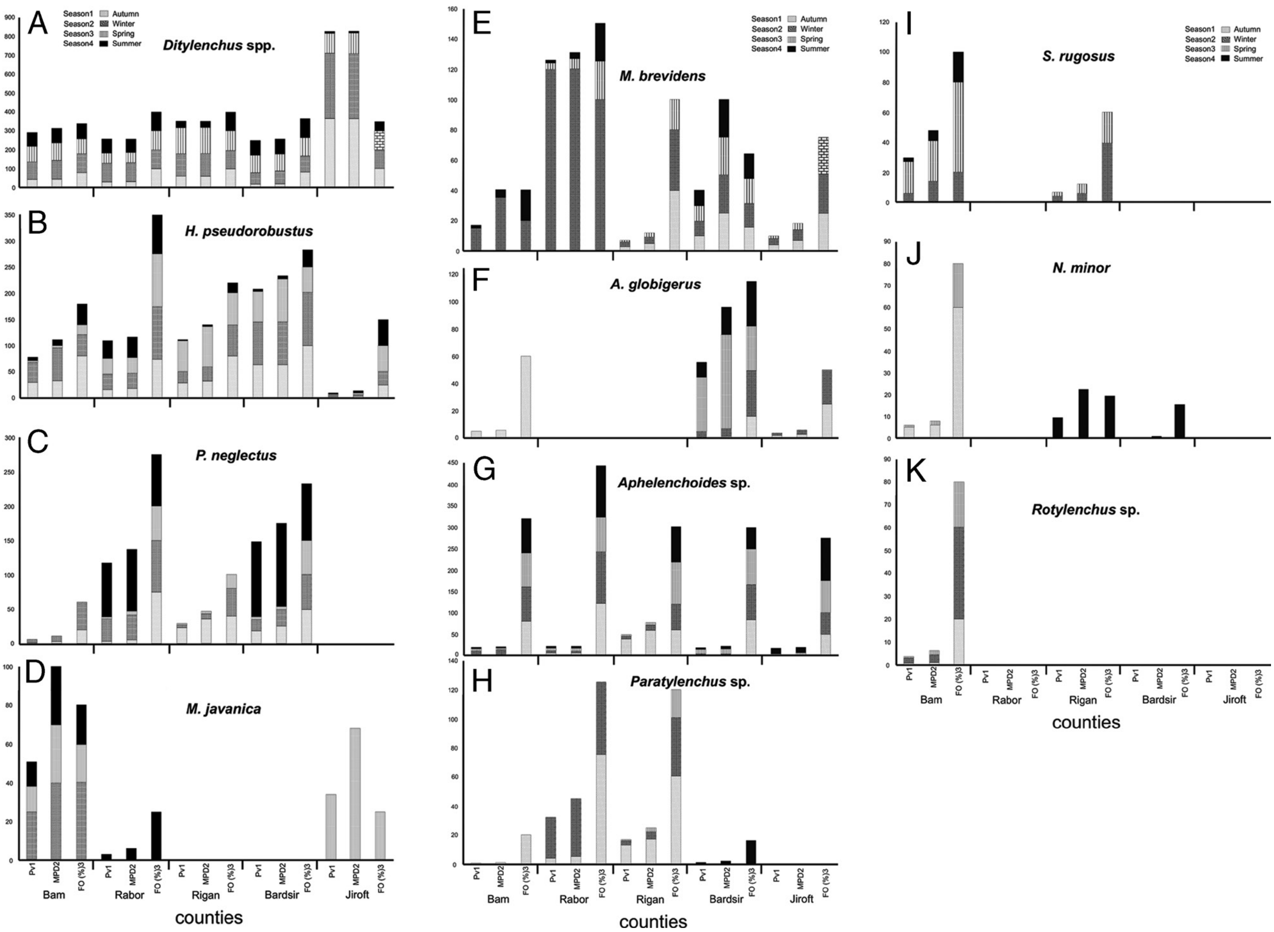

Figure 2: (A-K) Prominence, mean population densities and occurrence of 11 plant-parasitic nematode species identified from the bulk soil of alfalfa plants in five counties during four consecutive seasons (Autumn; Winter; Spring; Summer) in the Kerman Province of Iran.

Table 2. MANOVA of effect of the locality and season on nematode community using SPSS 24.

Source	Type III sum of squares	df	Mean square	*F*	Sig.
Corrected model	596,437.917[a]	19	31,391.469	423.827	0.000
Intercept	3827,900.417	1	3827,900.417	51,681.824	0.000
Location	45,860.167	4	11,465.042	154.794	0.000
Season	166,211.917	3	55,403.972	748.028	0.000
Location × Season	384,365.833	12	32,030.486	432.455	0.000
Error	2,962.667	40	74.067		
Total	4427,301.000	60			
Corrected Total	599,400.583	59			

[a]$R^2 = 0.995$ (Adjusted $R^2 = 0.993$).

Table 3. Prominence values (PV), mean population density (MPD) and frequency of occurrence (FO) (%) of plant-parasitic nematode species that were identified in association with alfalfa in five counties in the Kerman Province of Iran during four consecutive seasons (Fall, Winter, Spring and Summer) of 2013.

	Bam			Rabor			Rigan			Bardsir			Jiroft		
Species	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)
Ditylenchus spp.[a]															
Season 1	43	48	80	30	30	100	60	60	100	19	21	83	366	366	100
Season 2	94	94	100	103	103	100	120	120	100	59	65	83	344	344	100
Season 3	83	93	80	53	53	100	137	137	100	95	95	100	107	107	100
Season 4	71	79	80	72	72	100	36	36	100	76	76	100	8	11	50
H. pseudorobustus															
Season 1	29	32	80	16	18	75	29	32	80	64	64	100	2	3	25
Season 2	40	64	40	29	29	100	21	27	60	81	81	100	2	3	25
Season 3	2	4	20	31	31	100	60	78	60	59	84	50	3	4	50
Season 4	7	11	40	33	38	75	1	3	20	3	5	33	3	4	50
P. neglectus															
Season 1	1	3	20	4	5	75	23	36	40	18	25	50	0	0	0
Season 2	5	8	40	31	36	75	4	7	40	17	24	50	0	0	0
Season 3	0	0	0	4	6	50	2	4	20	4	6	50	0	0	0
Season 4	0	0	0	78	90	75	0	0	0	109	120	83	0	0	0
M. javanica															
Season 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Season 2	25	40	40	0	0	0	0	0	0	0	0	0	0	0	0
Season 3	13	30	20	0	0	0	0	0	0	0	0	0	34	68	25
Season 4	13	30	20	3	6	25	0	0	0	0	0	0	0	0	0
M. brevidens															
Season 1	0	0	0	0	0	0	3	5	40	10	25	16	4	7	25
Season 2	15	35	20	120	120	100	3	4	40	10	25	16	4	7	25
Season 3	0	0	0	4	7	25	1	3	20	10	25	16	2	4	25
Season 4	2	5	20	2	4	25	0	0	0	10	25	16	0	0	0

A. globigerus															
Season 1	5	6	60	0	0	0	0	0	0	0.5	1	16	2	3	25
Season 2	0	0	0	0	0	0	0	0	0	4	6	33	2	3	25
Season 3	0	0	0	0	0	0	0	0	0	40	69	33	0	0	0
Season 4	0	0	0	0	0	0	0	0	0	11	20	33	0	0	0
Aphelenchoides sp.															
Season 1	1	1	80	7	7	100	3	4	60	1	1	83	1	2	50
Season 2	11	12	80	4	4	100	1	2	60	1	1	83	1	2	50
Season 3	4	4	80	7	7	100	15	15	100	12	14	83	2	3	75
Season 4	4	4	80	5	5	100	7	8	80	5	7	50	13	13	100
Paratylenchus sp.															
Season 1	0.5	1	20	4	5	75	13	17	60	0	0	0	0	0	0
Season 2	0	0	0	28	40	50	3	5	40	0	0	0	0	0	0
Season 3	0	0	0	0	0	0	1	3	20	0	0	0	0	0	0
Season 4	0	0	0	0	0	0	0	0	0	1	2	16	0	0	0
S. rugosus															
Season 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Season 2	6	14	20	0	0	0	4	6	40	0	0	0	0	0	0
Season 3	21	27	60	0	0	0	3	6	20	0	0	0	0	0	0
Season 4	3	7	20	0	0	0	0	0	0	0	0	0	0	0	0
N. minor															
Season 1	5	6	60	0	0	0	0	0	0	0	0	0	0	0	0
Season 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Season 3	1	2	20	0	0	0	0	0	0	0	0	0	0	0	0
Season 4	0	0	0	0	0	0	10	23	20	0.5	1	16	0	0	0
Rotylenchus sp.															
Season 1	0.5	1	20	0	0	0	0	0	0	0	0	0	0	0	0
Season 2	2	3	40	0	0	0	0	0	0	0	0	0	0	0	0
Season 3	1	2	20	0	0	0	0	0	0	0	0	0	0	0	0
Season 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Including *D. acutus, D. myceliophagus, D. terricolus* and *D. sarvarae.*

Table 4. Prominence values (PV), mean population density (MPD) and frequency of occurrence (FO) (%) of plant-parasitic nematode species identified in association with alfalfa pooled for five counties in the Kerman Province of Iran and the four consecutive seasons (Fall, Winter, Spring and Summer) of 2013.

Nematode species	Prominence value (PV)	Mean population density (MPD)	Frequency of occurrence (FO: %)
Ditylenchus spp.	89	93	92
Helicotylenchus pseudorobustus	18	24	59
Pratylenchus neglectus	18	30	34
Meloidogyne javanica	10	41	6
Merlinius brevidens	8	18	21
Amplimerlinius globigerus	6	18	12
Aphelenchoides sp.	4	6	41
Paratylenchus sp.	3	8	16
Scutylenchus rugosus	3	12	7
Nanidorus minor	2	6	11
Rotylenchus sp.	1	3	7

alfalfa in the Kerman Province according to PV were *Ditylenchus* spp. (e.g. *D. acutus*, *D. myceliophagus*, *D. terricolus* and *D. sarvarae*), followed by *H. pseudorobustus*, *P. neglectus* and *M. javanica*, whereas the least dominant was *Rotylenchus* sp. (Table 4). *Ditylenchus* spp. had the highest occurrence, whereas *Rotylenchus* sp. and *S. rugosus* occurred least among the samples.

Nematode biodiversity indices (H' or E) differed significantly ($p \leq 0.05$) among counties (Table 5), where H' and E values were highest in Bam and lowest in Jiroft. A significant, negative correlation existed for soil pH and the abundance of *S. rugosus* ($r = -0.964$; $p \leq 0.01$), whereas significant and positive correlations existed between soil EC and the abundance of *H. pseudorobustus* ($r = 0.89$; $p \leq 0.05$), *Aphelenchoides* sp. ($r = 0.92$; $p \leq 0.05$), *A. globigerus* ($r = 0.97$; $p \leq 0.01$) and *P. neglectus* ($r = 0.90$; $p \leq 0.05$) (Table 6). The PCA was performed to study the correlation of the temperature and rainfall with the counties (Fig. 3). An accumulated variability of 94.62% was detected in the analysis which was 59.53% for F1 and 35.09% for F2. The active variables including rainfall (–0.83) and EC (–0.85) had negative correlation to F1, whereas temperature (0.98) and pH (0.12) had positive correlation to F1. Rainfall (–0.44) and temperature (–0.05) had negative correlation to F2, whereas EC (0.52) and pH (0.97) had positive correlation to F2. The results suggested that in Bardsir county EC affected diversity of plant-parasitic nematodes more than in other counties, whereas in

Table 5. Nematode community indices for the five counties sampled during four consecutive seasons in the Kerman Province of Iran during 2013.

County	Shannon index (H')	Evenness index
Bam	2.4 ± 0.02	0.83 ± 0.02
Bardsir	2.2 ± 0.03	0.73 ± 0.03
Jiroft	1.0 ± 0.01	0.58 ± 0.01
Rabor	2.1 ± 0.02	0.74 ± 0.02
Rigan	2.1 ± 0.02	0.71 ± 0.02

Ranges are mean ± standard error.

Table 6. Correlation data for pH and electrical conductivity (EC) and plant-parasitic nematode species[a] identified in association with alfalfa from five counties in the Kerman Province of Iran during 2013.

	Helicotylenchus	*Ditylenchus*	*Amplimerlinius*	*Pratylenchus*	*Aphelenchoides*	*Rotylenchus*	*Nanidorus*	*Paratylenchus*	*Scutylenchus*	*Merlinius*	*Meloidogyne*	pH	EC
Helicotylenchus	1	–0.334	0.838	0.764	0.941*	0.111	–0.156	–0.101	–0.045	–0.114	–0.528	0.1	0.885*
Ditylenchus	–0.334	1	0.122	–0.318	–0.113	–0.689	0.212	–0.638	–0.341	–0.58	0.259	0.282	–0.06
Amplimerlinius	0.838	0.122	1	0.783	0.872	0	–0.388	–0.355	–0.295	–0.15	–0.361	0.236	0.972**
Pratylenchus	0.764	–0.318	0.783	1	0.799	0.276	–0.584	0.294	–0.501	0.426	–0.674	0.44	0.902*
Aphelenchoides	0.941*	–0.113	0.872	0.799	1	–0.159	–0.067	–0.069	–0.349	–0.171	–0.7	0.411	0.917*
Rotylenchus	0.111	–0.689	0	0.276	–0.159	1	–0.747	0.274	0.433	0.703	0.287	–0.571	0.083
Nanidorus	–0.156	0.212	–0.388	–0.584	–0.067	–0.747	1	–0.143	0.139	–0.706	–0.104	0.097	–0.435
Paratylenchus	–0.101	–0.638	–0.355	0.294	–0.069	0.274	–0.143	1	–0.359	0.781	–0.568	0.398	–0.128
Scutylenchus	–0.045	–0.341	–0.295	–0.501	–0.349	0.433	0.139	–0.359	1	–0.253	0.688	–0.964**	–0.368
Merlinius	–0.114	–0.58	–0.15	0.426	–0.171	0.703	–0.706	0.781	–0.235	1	–0.194	0.108	0.027
Meloidogyne	–0.528	0.259	–0.361	–0.674	–0.7	0.287	–0.104	–0.568	0.688	–0.194	1	–0.802	–0.522
pH	0.1	0.282	0.236	0.44	0.411	–0.571	0.097	0.398	–0.964**	0.108	–0.802	1	0.322
EC (mhos/cm)	0.885*	–0.06	0.972**	0.902*	0.917*	0.083	–0.435	–0.128	–0.368	0.027	–0.522	0.322	1

[a]The species including *H. pseudorobustus; Ditylenchus* spp. *(D. acutus, D. myceliophagus; D. terricolus, D. sarvarae); A. globigerus; P. neglectus; N. minor; S. rugusous; M. javanica; Aphelenchoides* sp.; *Rotylenchus* sp. *and Paratylenchus* sp. * = 95%, ** = 99%.

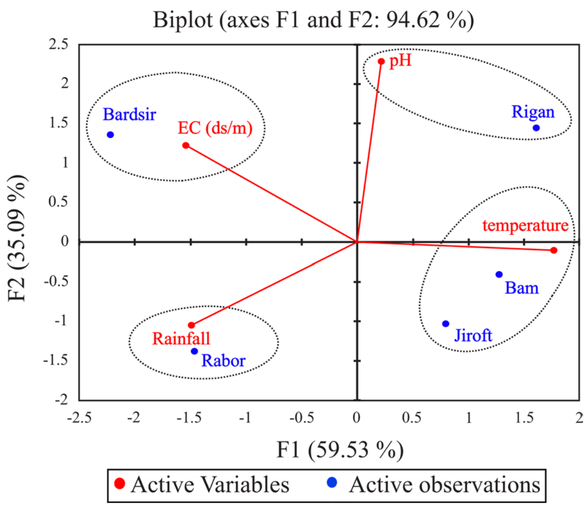

Figure 3: Correlation of the temperature, rainfall, pH and EC on the diversity of plant-parasitic nematodes for the counties (Bam, Rabor, Rigan, Bardsir and Jiroft) using principal component analysis (PCA).

Rigan county pH was the most effective factor on nematode diversity. In the counties Jiroft and Bam, temperature played an important role, whereas in Rabor county rainfall played an important role in the diversity of plant-parasitic nematodes (Fig. 3).

Discussion

The study provided baseline information on plant-parasitic nematode diversity associated with alfalfa bulk soil in the south eastern, Kerman Province of Iran. Among the identified 11 plant-parasitic nematode genera and 12 species, the predominant genera in descending order were *Ditylenchus*, *Helicotylenchus*, *Pratylenchus* and *Meloidogyne*, which have also been recorded in alfalfa cultivated in the East Azarbaijan Province of Iran (Alavi and Barooti, 1995; Eskandari et al., 2015) and other countries (Goodell and Ferris, 1980; Marais, 1990; Westerdahl and Frate, 2007; McCord, 2012). Interestingly, the predominant genus in the soil, *Ditylenchus*, with identification of a new species *D. savarae* (Shokoohi et al., 2018), was associated with alfalfa in the Kerman Province. *Helicotylenchus pseudorobustus*, second in predominance, was reported as the most abundant in alfalfa fields in Colorado, USA (Simmons et al., 2008), in Oman, South Africa and other countries (Marais, 1990; Mani and AL Hinai, 1996). *Pratylenchus neglectus*, as the third predominant genus identified in the current study, was the most commonly occurring genus associated with alfalfa in Oman (Mani and Al Hinai, 1996) and has also been reported in South Africa and other countries (Marais, 1990; Simmons et al., 2008). *Meloidogyne javanica*, generally low in abundance in the soil of alfalfa in our study except for Jiroft, being fourth in predominance, was also reported as abundant in alfalfa fields in Oman (Mani and Al Hinai, 1996) and South Africa (Marais, 1990). *Merlinius brevidens*, fifth in predominance, also occurred in the soil samples from all counties sampled in the Kerman Province and has been reported from alfalfa fields in other countries (Westerdahl and Frate, 2007).

The production area under investigation during our study covered 180 726 km^2 with different abiotic factors such as climate (e.g. temperature and rainfall) (Ferris et al., 2012; Hashemi Nasab Khabisi et al., 2013; Kavian et al., 2016), elevation (Kergunteuil et al., 2016) and edaphic variables (Sarreshtehdari, 2002), which could impact the nematode assemblages. Such factors are major determinants of survival and reproduction of plant-parasitic nematodes since they affect nematode occurrence, population densities and the degree of symptom development and expression in infected hosts (Amarasena et al., 2016). Seasonal fluctuations of nematode population densities are common in alfalfa ecosystems, varying among localities and over years (Norton, 1963; Williams-Woodward and Gray, 1999; Simmons et al., 2008). The plant-parasitic nematode diversity associated with alfalfa in the Iranian counties over four consecutive seasons suggested that the environmental conditions played an important role in nematode ecology. This phenomenon was most pronounced for the abundance of *Merlinius*, showing a significant interaction for county × season for *Merlinius* and *Pratylenchus* among the five counties, and for *Merlinius* and *Ditylenchus* for seasons. Although no correlation statistics was done for climatic variables during our study due to such data not being recorded at each field for each county, the seasonal effect was most probably one of the major contributors to the predominance of the genera at localities. This was premised on the basis that climatic conditions in the province ranged from dry and cold (Rabor and Bardsir counties) to warm and humid (Bam, Jiroft and Rigan counties) (Jalali-Far et al., 2012). For example, data from our study showed that during winter *Helicotylenchus pseudorobustus* and *Merlinius brevidens* dominated at Bardsir and Rabor when minimum temperatures lower than 5°C prevailed (Hashemi Nasab Khabisi et al., 2013;

Kavian et al., 2016). Nonetheless, for *Helicotylenchus* these findings contradicted reports stating that population densities of the genus decreased at 5 to 10°C, with optimum temperature for development, reproduction and survival being from 20 to 30°C (Azmi, 1979; Nath et al., 1998). Similarly, *Merlinius* also prefers moderate temperatures of 20°C (Malek, 1980), which is in contrast to the predominance of this genus in winter at Bardsir and Rabor, where temperatures lower than 5°C prevailed. *Pratylenchus neglectus* on the other hand was dominant in summer at Bardsir at a higher mean temperature within 25 and 30°C being preferred by species of this genus to support their optimal biological functions (Griffin and Gray, 1990; Mizukubo and Adachi, 1997). Dominance of *Ditylenchus* species in Jiroft during Autum and Winter and *M. javanica* during Spring again accentuated the preference of species of these genera to optimally develop and reproduce at relatively mild temperature ranges and in areas with relatively high rainfall (Loubser and Meyer, 1987; Morris et al., 2011; Hajihassani, 2016; Hajihassani et al., 2017). The absence of genera at some localities and during some seasons, e.g. *Meloidogyne* species not occurring at Bardsir (low minimum and mild to high maximum temperatures) and Rigan (mild to high temperature range), and not during Autumn at all five localities, is another phenomenon emanating from our study for which an explanation could not be given at this stage, and warrants further investigation. The PCA also showed the diversity of nematode in Rabor as being correlated with rainfall, which is in accordance with the rainfall value of the county. This result is in agreement with Munteanu (2017), which indicated that rainfall and temperature positively affected the diversity of nematodes in Norway.

Concerning soil texture, significant, positive correlations recorded between the abundance of *H. digonicus* and *M. brevidens* in alfalfa fields in California (USA) and fine-textured soils, while that of *Meloidogyne arenaria* showed negative but significant correlations with this soil type (Goodell and Ferris, 1980). However, no deductions can be made on this topic from our study, but it is interesting that *H. pseudorobustus* and *Merlinius brevidens* were dominant in sandy-loam soils in Bardsir and Rabor, and *Meloidogyne javanica* in sandy-loam soils in Jiroft.

The biodiversity indices H’ and E, which are popular and useful tools for studying different factors and their effects on nematode populations, revealed seasonal effects regarding the diversity of the plant-parasitic nematode genera identified as a result of our study. A higher H’ and E value, for nematode assemblages from alfalfa fields in the Bam, and a slightly lower value for the Jiroft accentuated the effects that different abiotic and/or biotic factors may have on nematode assemblages. These indices, however, varied among the counties suggesting that a greater diversity and more even distribution of the prevalent nematode genera and species occurred at Bam, Bardsir, Rabor and Rigan compared to Jiroft. This scenario especially applies for the H’ index, which is suggested to be used in Iran.

High levels of the selected soil chemical properties determined in our study, namely, pH and EC, have been reported to result in decreased population densities of plant-parasitic nematode populations. No correlation was apparent for soil pH and nematode genera identified during our study, except for *Scutylenchus* with a high negative correlation with pH, since values generally ranged in the neutral zone (7.51–7.59) (McCauley et al., 2017) for the other genera. Matute (2013), however, reported that pH levels between 5 and 6 negatively correlated with plant-parasitic nematodes abundance in the rhizosphere of *Brassica rapa* in central Arkansas (USA). In addition, Burns (1971) reported significantly higher abundance of Tylenchinae and *Hoplolaimus galeatus*, associated with soybean in the USA, at pH 6 than at pH 4 or 8. Concerning EC, significant correlations for *Helicotylenchus* and *Pratylenchus* and this variable is in agreement with results by Mendoza et al. (2008). These authors showed a positive correlation between soil EC and low nematode abundance in Nebraska (USA). It is important to note that other soil chemical properties such as calcium, iron, organic matter and nitrogen (Mateille et al., 2014) could have impacted on nematode population densities but were not determined during our study and should be included in future research on this topic.

Conclusion

County Bam, with a higher quality of alfalfa (Tadayyon and Zafarian, 2016), also had the highest plant-parasitic nematode diversity, and therefore, crop production practices known to promote high plant-parasitic diversity should be practiced. Environmental conditions were shown to affect the diversity of plant-parasitic nematodes in Kerman Province, as shown by the PCA, but detailed studies on this topic are needed. Breeding of alfalfa cultivars with resistance to economically important nematode pests such as *Meloidogyne* and *Pratylenchus* species should be considered to protect alfalfa crops in Kerman Province. Future studies should also establish whether economically important nematodes of aerial parts on alfalfa are present in the province.

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References

Abivardi, C. and Sharafeh, M. 1973. The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn 1857) Filipjev 1936 as an important threat for cultivation of alfalfa in Iran. Nematologia Mediterranea 1:22–7.

Addinsoft. 2007. XLSTAT, Analyse de données et statistique avec MS Excel, Addinsoft, New York, NY.

Alavi, A. and Barooti, S. H. 1995. Plant Nematology, Fundamental and Quarantine Nematodes of Iran, Plant Disease and Pest Institute Publisher, Tehran.

Amarasena, P. G. D. S., Mohotti, K. M. and De Costa, D. M. 2016. Effects of changing rainfall and soil temperature on population density of *pratylenchus loosi* in tea lands at different elevations. Tropical Agricultural Research 27:265–76.

Andrássy, I. 2005. Free-living nematodes of Hungary (Nematoda errantia), Vol. I. Budapest, Hungrian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences, Budapest.

Azmi, M. I. 1979. Responses to temperature in nematodes 1. Mechanism of heat tolerance in *helicotylenchus dihystera*. Nematologia Mediterranea 7:163–75.

Beretta, A. N., Silbermann, A. V., Paladino, L., Torres, D., Bassahun, D., Musselli, R. and García-Lamohte, A. 2014. Soil texture analyses using a hydrometer: modification of the Bouyoucos method. Ciencia e Investigación Agrarian 41:263–71.

Biederman, L. A. and Boutton, T. W. 2009. Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland. Soil Biology and Biochemistry 41:1943–50.

Bolton, C., De Waele, D. and Loots, G. C. 1989. Plantparasitic nematodes on field crops in South Africa. 3. Sunflower. Revue de Nematologie 12:69–76.

Bongers, T. 1990. The maturity index an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14–9.

Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analysis of soils. Agronomy Journal 54:464–5.

Brzeski, M. W. 1991. Review of the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Anguinidae). Revue de Nématologie 14:9–59.

Burns, N. C. 1971. Soil pH effects on nematode populations associated with soybeans. Journal of Nematology 3:238–45.

Castillo, P. and Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management, in Hunt, D. J. and Perry, R. N. (Eds), Nematology Monographs and Perspectives, Vol. 6. Brill Leiden, Boston, MA, 530pp.

Colwell, R. K. 2009. Biodiversity: concepts, patterns, and measurement, in Levin, S.A. (Ed.), The Princeton Guide to Ecology, Princeton University Press, Princeton, NJ, pp. 257–63.

De Grisse, A. 1969), Redescription ou modifications de quelques techniques utililisées dans l'étude des nématodes phytoparasitaires. Mededelingen van de Rijksfaculteit Landbouwetenschappen Gent 34:351–69.

De Waele, D. and McDonald, A. H. 2000. Diseases caused by nematodes, in Frederiksen, R. A. and Odvody, G. N. (Eds), Compendium of Sorghum Diseases, American Phytopathological Society, St Paul, MN, pp. 50–3.

Eskandari, S. H., Niknam, G. H. and Karegar, A. 2015. Identification of the plant parasitic nematodes in the alfalfa fields of Ahar (East Azerbaijan Province). In Persian, Plant Protection 38:1–11.

Ferris, H., Griffiths, B. S., Porazinska, D. L., Powers, T. O., Wang, K. H. and Tenuta, M. 2012. Reflections on plant and soil nematode ecology: past, present and future. Journal of Nematology 44:115–26.

Geraert, E. 2008. The Tylenchidae of the World: Identification of the Family Tylenchidae (Nematoda), Academia Press, Gent.

Geraert, E. 2011. The Dolichodoridae of the World: Identification of the Family Dolichodoridae (Nematoda), Academia Press, Gent.

Ghanavati, F., Mozaffari, J., Masoumi, A. and Kazempour, S. 2007. Morphological studies of pollen grains of *Medicago* species in Iran. Journal of Crop Science 34:184–99.

Goodell, P. B. and Ferris, H. 1980. NEMASAM-A sample simulator for five plant-parasitic nematodes. Journal of Nematology 12:222.

Gray, F. A. and Griffin, G. D. 1994. Plant parasitic nematodes of alfalfa in the United States. Journal of Nematology 26:705–19.

Gray, F. A., Williams, J. L., Griffin, G. D. and Wilson, T. E. 1994. Distribution in the western United States on alfalfa and cultivar reaction to mixed populations of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi*. Journal of Nematology 26:705–19.

Griffin, G. D. and Gray, F. A. 1990. Biology and pathogenicity of *Pratylenchus neglectus* on alfalfa. Journal of Nematology 22:546–51.

Hafez, S. L. 1998. Alfalfa nematodes in Idaho. Current information series No. 806, University of Idaho, Idaho.

Hafez, S. L. and Sundararaj, P. 2009. Nematodes of alfalfa and management. Proceedings, Western Alfalfa and Forage Conference, 2–4.

Hajihassani, A. 2016. Studies of plant host preferences of the stem nematodes, *Ditylenchus weischeri* and *D. dipsaci*. PhD thesis, University of Manitoba, Winnipeg, 158pp.

Hajihassani, A., Tenuta, M. and Gulden, R. H. 2017. Griffin, G. D., and F. A. Gray. 1990. Biology and pathogenicity of *Pratylenchus neglectus* on alfalfa, Journal of Nematology 22:546–51.

Hashemi Nasab Khabisi, F., Mosavi Bayegi, M., Bakhtiari, B. and Davari, K. 2013. Prediction the rainfall changes with downscaling LARS-WG and HadCM3 models in Kerman during the next 20 years (2030–2011). Irrigation and Water Engineering 3:43–58.

Hassanzadeh, Z., Karegar, A. and Kheiri, A. 2004. Identification of the nematodes of the order Tylenchida in the alfalfa fields of the Hamedan Province. Proceeding of the 16th Plant Protection Congress, Tabriz, pp. 127.

IBM. 2016. IBM SPSS Statistics for Windows, Version 24.0. IBM Corp, Armonk, NY.

Jalali-Far, M., Kalantari, M. H., Naderi, M., Moradi, M., Pour-Dehqan, D., Dahesh, K. H., Latif-Kar, M., Naderi, A. A. and Rouholamini, F. B. 2012. Province of Kerman. Ministry of Education Publishing, Kerman.

Kavian, A., Jafarian, Z., Jahanshahi, A. and Golshan, M. 2016. Land erosion mapping of precipitation using irrigated land statistics in Kerman province. Natural Geographical Research 48:51–68.

Kergunteuil, A., Campos-Herrera, R., Sánchez-Moreno, S., Vittoz, P. and Rasmann, S. 2016. The abundance, diversity, and metabolic footprint of soil nematodes Is highest in high elevation alpine grasslands. Frontier in Ecology and Evolution 4:84.

Kheiri, A. 1972. Plant parasitic nematodes (Tylenchida) from Iran. Biologisch Jaarboek Dodonaea 40:224–39.

Kleynhans, K. P. N., Van Der Berg, E., Swart, A., Marais, M. and Buckley, N. H. 1996. Plant Nematodes in South Africa. Handbook No. 8. Government Printers, Pretoria.

Loubser, J. T. and Meyer, A. J. 1987. Population Dynamics of the Root-knot Nematodes *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood on Grapevines in two different Regions of South Africa. South African Journal of Enology and Viticulture 8:36–40.

McCauley, A., Jones, C. and Olson-Rutz, K. 2017. Soil pH and Organic Matter. Nutrient Management Module No. 8. Montana State University, Montana, pp. 1–16.

McCord, P. H. 2012. Relationship of Resistance to *Meloidogyne chitwoodi* (race 2) and *M. hapla* in Alfalfa. Journal of Nematology 44:387–90.

Malek, R. B. 1980. Population Response to Temperature in the Subfamily Tylenchorhynchinae. Journal of Nematology 12:1–6.

Mani, A. and Al Hinai, M. S. 1996. Plant-parasitic nematodes associated with alfalfa and fluctuations of *Pratylenchus jordanensis* population in the Sultanate of Oman. Fundamental and Applied Nematology 20:443–7.

Marais, M. 1990. Plant-parasitic nematodes in lucerne fields in South Africa. Phytophylactica 22:449–52.

Mateille, T., Tavoillot, J., Martiny, B. and Fargette, M. 2014. Importance of soil characteristics for plant-parasitic nematode communities in European coastal foredunes. European Journal of Soil Biology 64:53–60.

Matute, M. M. 2013. Soil Nematodes of *Brassica rapa*: Influence of Temperature and pH. Advances in Natural Science 6:20–26.

Mendoza, R. B., Franti, T. G., Doran, J. W., Powers, T. O. and Zanner, C. W. 2008. Tillage effects on soil quality indicators and nematode abundance in loessial soil under long-term no-till production. Communications in Soil Science and Plant Analysis 39:2169–90.

Milano de Tomasel, M. C. and McIntyre, G. A. 2001. Distribution and biology of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in alfalfa grown in Colorado. Nematropica 31:11–6.

Mizukubo, T. and Adachi, H. 1997. Effect of temperature on *pratylenchus penetrans* development. Journal of Nematology 29:306–14.

Morris, K. S., Horgan, F. G., Downes, M. J. and Griffin, C. T. 2011. The effect of temperature on hatch and activity of second-stage juveniles of the root-knot nematode, *Meloidogyne minor*, an emerging pest in north-west Europe. Nematology 13:985–93.

Munteanu, R. 2017. The effects of changing temperature and precipitation on free-living soil nematoda in Norway. Lund University, Lund, Norway, 30pp.

Nath, R. C., Mukherjee, B. and Dasgupta, M. K. 1998. Population behaviour of *Helicotylenchus multicinctus* in soil and roots of banana in Tripura, India. Fundamental and Applied Nematology 21:353–8.

Neher, D. A., Wu, J., Barbercheck, M. E. and Anas, O. 2005. Ecosystem type affects interpretation of soil nematode community measures. Applied Soil Ecology 30:47–64.

Norton, D. C. 1963. Population fluctuations of *Xiphinema americanum* in Iowa. Phytopathology 53:66–8.

Pen-Mouratov, S., Shukurov, N. and Steinberger, Y. 2010. Soil freeliving nematodes as indicators of both industrial pollution and livestock activity in Central Asia. Ecological Indicator 10:955–67.

Potter, J. W. and McKeown, A. W. 2003. Nematode biodiversity in Canadian agricultural soils. Canadian Journal of Soil Science 83:289–302.

Rowell, D. L. 1994. Soil Science: Methods and Applications, Longman Group, Harlow.

Sarreshtehdari, A. 2002. The impact of a flood spreading project on soil properties, a case study in Iran, Kerman Province, Bam, Abbarik. MSc thesis, Institute for Geo-Information Science & Earth Observation (ITC), Enschede, The Netherlands.

Shokoohi, E., Iranpour, F., Peneva, V., Elshishka, M., Fourie, H. and Swart, A. 2018. *Ditylenchus sarvarae* sp. n. (Tylencina: Anguinidae) from Iran. Zootaxa 4399:198–204.

Shokoohi, E., Iranpour, F., Swart, A., Fourie, H. and Panahi, H. 2018. Morphological and molecular characters of three *Ditylenchus* species from Iran. Tropical Zoology 161–76.

Simmons, B. L., Niles, R. K. and Wall, D. H. 2008. Distribution and abundance of alfalfa-field nematodes at various spatial scales. Applied Soil Ecology 38:211–22.

Spaull, V. W. and Braithwaite, J. M. C. 1979. A comparison of methods for extracting nematodes from soi1 and roots of sugarcane. Proceeding South African Sugarcane Technology 53:103–7.

Straube, D. and Juen, A. 2013. Storage and shipping of tissue samples for DNA analyses: A case study on earthworms. European Journal of Soil Biology 57:13–8.

Sturhan, D. and Brzeski, M. W. 1991. Stem and Bulb Nematodes, *Diylenehus* spp. Manual of Agricultural Nematology Marcel Dekker, New York, NY, pp. 423–64.

Tadayyon, A. and Zafarian, M. 2016. Effect of humic acid on some agronomic characters of some varieties of Alfalfa (*Medicago sativa* L.), in Persian with English abstract. Journal of Crop Ecophysiology 10:85–598.

Tucak, M., Popovic, S., Cupic, T., Grljusic, S., Bolaric, S. and Kozumplikn, V. 2008. Genetic diversity of alfalfa (*Medicago* spp.) estimated by molecular markers and morphological characters. Periodicum Biologorum 110:243–9.

Wang, K. H., McSorley, R. and Gallaher, R. N. 2009. Can nematode community indices elucidate plant health conditions? Journal of Nematology 41:392.

Westerdahl, B. B. and Frate, C. 2007. Parasitic nematodes in alfalfa, in Summers, C. G. and Putnam, D. H. (Eds), Irrigated Alfalfa Management for Mediterranean and Desert Zones, UC ANR 3512, Oakland.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55:25–38.

Williams-Woodward, J. L. and Gray, F. A. 1999. Season fluctuations of soil and tissue populations of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in alfalfa. Journal of Nematology 31:27–36.

Yeates, G. W. 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils 37:199–210.

Zeng, Y., Ye, W., Martin, B., Martin, M. and Tredway, L. 2012. Diversity and occurrence of plant-parasitic nematodes associated with golf course turfgrasses in North and South Carolina, USA. Journal of Nematology 44:337–47.

Zhang, M., Liang, W. J. and Zhang, X. K. 2012. Soil nematode abundance and diversity in different forest types at Changbai Mountain, China. Zoological Studies 51:619–26.